

Changes in Larval Morphology in the Evolution of Benthic Development by *Patiriella exigua* (Asteroidea: Asterinidae), a Comparison with the Larvae of *Patiriella* Species with Planktonic Development

M. BYRNE

*Department of Anatomy and Histology, F-13, University of Sydney,
New South Wales 2006, Australia*

Abstract. *Patiriella exigua* (Lamarck) is a small asterinid sea star that deposits large eggs onto the substratum. Development is lecithotrophic and entirely benthic, proceeding without parental care. The embryos develop to the brachiolaria stage before hatching and there is no trace of a bipinnaria larva. In its early stage the larva of *P. exigua* resembles a planktonic brachiolaria in having one long central brachium and two short lateral brachia. By hatching, the brachia are equal in length, giving the larva a tripod-like appearance. Comparison of the larva of *P. exigua* with the brachiolaria of *Patiriella* species with planktonic development supports the hypothesis that the tripod larval form results from differential growth of the lateral brachia. At hatching, the *P. exigua* larva has a well-developed attachment complex composed of a large adhesive disk and three muscular brachia; the latter bear a striking resemblance to adult tube feet. This hypertrophic elaboration of the brachiolar complex is an adaptation for permanent benthic attachment. Internally, one large enterocoel forms at the anterior end of the archenteron. The archenteron then closes to form the rudiment for the adult gut. Metamorphosis involves gradual resorption of the brachiolar complex concomitant with formation of the first tube feet. The adhesive disk plays a major role in attachment during late metamorphosis but is gradually reduced to a plug of tissue as the tube feet become functional. Juvenile *P. exigua* are negatively geotactic and float on the water surface, behavior that may act as a mechanism for dispersal. The similarity of the early larva of *P. exigua* to planktonic brachiolariae suggests that the evolution of benthic lecithotrophy by this species involved

modification of a planktonic larval form. These modifications include elimination and reduction of larval feeding structures, formation of one rather than three enterocoels, and hypertrophy of the brachiolar complex to form a tripod larva. Heterochronies in the ontogeny of *P. exigua* include the delay in hatching to the brachiolaria stage and the accelerated development of the juvenile form and adult skeleton.

Introduction

The sea star *Patiriella exigua* (Lamarck) is a conspicuous component of the intertidal echinoderm fauna of southeastern Australia and is a member of a sympatric group of *Patiriella* species distributed around the south coast from Western Australia to north Queensland (Dartnall, 1971; Keough and Dartnall, 1978; Lawson-Kerr and Anderson, 1978; Byrne, 1991, 1992; Byrne and Barker, 1991). This genus of sea stars exhibits a striking diversity of life histories that span the range of developmental patterns seen in the Asteroidea. *P. regularis* shows what is considered to be the ancestral pattern for asteroids (Strathmann, 1978), developing through planktotrophic bipinnaria and brachiolaria larvae (Byrne and Barker, 1991). In contrast, the other *Patiriella* species have modified ontogenies. *P. gummii*, *P. calcar*, *P. brevispina*, and *P. pseudoexigua* develop through lecithotrophic planktonic brachiolariae, whereas *P. exigua* develops through a lecithotrophic benthic brachiolaria (Lawson-Kerr and Anderson, 1978; Byrne, 1991, 1992; Chen and Chen, 1992). *P. vivipara* and *P. parvivipara* show the most modified development, having reduced intragonadal larvae

and giving birth to juveniles (Dartnall, 1971; Keough and Dartnall, 1978; Byrne, 1991; Chia and Walker, 1991). These congeneric species, which occupy similar habitats and latitude and which have such developmental diversity, are a model system with which to examine the evolutionary modification of larval form because of the ease with which homologous structures can be identified and compared (Byrne, 1991; Byrne and Barker, 1991). The planktotrophic development of *P. regularis* (Byrne and Barker, 1991) provides the basis against which the ontogeny of the lecithotrophic developers, including *P. exigua*, can be compared (Lawson-Kerr and Anderson, 1978; Byrne, 1991).

P. exigua is a small cushion-shaped asterinid sea star (maximum R = 15 mm) that releases large eggs (390 μm diameter) through orally directed gonopores (Lawson-Kerr and Anderson, 1978; Byrne, 1991, 1992). The eggs adhere to the substratum with their sticky jelly coat, and development proceeds without parental care. *P. exigua* is a hermaphrodite and occasionally lays what appear to be self-fertilized eggs (Byrne, 1991; Byrne and Anderson, 1994). Reproduction and aspects of the development of this species are described in several studies (Lawson-Kerr and Anderson, 1978; Byrne, 1991, 1992; Cerra and Byrne, 1995a, b).

External and internal morphogenesis in the larvae of *P. exigua* is documented in detail here through the use of light, confocal, and scanning electron microscopy. Unlike *Patiriella* species with planktonic larvae, *P. exigua* lacks a discrete settlement stage and the larvae do not exhibit rapid metamorphosis. The juvenile form in *P. exigua* is attained through a gradual transformation in morphology. Particular attention is paid to the larval specializations involved with benthic attachment and to the gradual resorption of these structures during metamorphosis. Initial examination of the brachiolaria of *P. exigua* suggested that benthic development in this species is associated with hypertrophic elaboration of the larval attachment complex (Byrne, 1991). The hypothesis that the robust brachia of *P. exigua* are the result of differential growth is examined here through comparison with the brachia of *Patiriella* species with planktonic development. The ontogeny of *P. exigua* is compared with that of *P. regularis*, *P. calcar*, and *P. gunnii* to determine the morphological and heterochronic changes associated with the evolution of benthic lecithotrophy. As applied here to *Patiriella*, the term benthic development refers strictly to the situation in which the developmental stages either adhere to the substratum or are brooded by the parent; it does not include unattached demersal larvae (see Janies and McEdward, 1993).

Materials and Methods

Patiriella exigua is distributed from northern New South Wales (28°39'S; 153°37'E) to the Eyre Peninsula

in South Australia (34°44'S; 135°52'E). Adult specimens and egg masses attached to the undersurface of small boulders were collected at low tide from an intertidal rock platform at Clovelly, Sydney (33°54'S; 151°17'E), between August and October 1990 and 1991. The boulders and attached egg masses were transferred to laboratory aquaria to document the development and behavior of the larvae. Development was also examined in fertilizations carried out in the laboratory. Mature ovaries were dissected from *P. exigua* and placed in dishes containing 10^{-5} M 1-methyladenine (1-MA) in filtered seawater to induce maturation and release of eggs (Kanatani, 1969). Upon release, the eggs adhered to the bottom of the dishes with their jelly coats. The ovarian tissue was removed and the eggs were rinsed by decanting the 1-MA solution followed by a wash in fresh seawater. Sperm obtained from mature males through dissection of the testes were stored at 4°C until used for fertilization. For fertilization, a few drops of diluted sperm were added to the culture chambers. After 5–10 min, the eggs were washed in fresh seawater to remove excess sperm and the embryos were cultured at 19–21°C.

Development of *P. exigua* was documented by light microscopy (LM), confocal microscopy, and scanning electron microscopy (SEM). Live specimens were photographed with a photomicroscope. For LM and SEM, the embryos and larvae were fixed in 2.5% glutaraldehyde in filtered seawater for 1 h at room temperature. The larvae were relaxed in 6.8% MgCl_2 in distilled water before being placed in the primary fixative. Following primary fixation, the specimens were washed in 2.5% NaHCO_3 (pH 7.2) and post-fixed in 2% OsO_4 in 1.25% NaHCO_3 for 1 h at room temperature. The specimens were then washed in distilled water and dehydrated in a graded series of ethanols. For LM sections, the larvae were embedded in Spurr's resin. Serial sections (0.5 μm) were stained with 1% toluidine blue in 0.5% ethanolamine. For SEM, the specimens were critical-point dried, sputter coated, and viewed with a JOEL JSM-35C scanning electron microscope.

For confocal microscopy, relaxed larvae were fixed in 2% paraformaldehyde in seawater. The larvae were then dehydrated in graded ethanols, cleared in Histo-Clear (National Diagnostics; Atlanta, Georgia), and mounted on slides. Optical sections of the specimens were made with a BIO-RAD MRC600 confocal microscope.

Development of the skeleton was documented by utilizing the birefringent properties of the calcite ossicles. The larvae are opaque, and whole mounts of early larvae fixed in glutaraldehyde, dehydrated in alcohol, and cleared in Histo-Clear were observed with polarized light. To examine development of the skeleton during metamorphosis, advanced larvae were fixed in glutaraldehyde and during dehydration were stained with 70% alcoholic eosin

for 1 h. Whole-mounts of these specimens were examined with bright-field illumination.

The arms and body lengths of the brachiolaria of *P. exigua* and several *Patiriella* species with planktonic development were measured to compare the development of the brachia. For *P. exigua*, live larvae were measured with an ocular micrometer. The arms and body lengths of the brachiolariae of *P. regularis*, *P. calcar*, and *P. gunnii* were measured from photographs of live larvae. These measurements were used to calculate the ratio of body to arm length for each species.

Results

External morphogenesis

The chronology of development of *Patiriella exigua* is outlined in Table I. By 24 h the embryos are gastrulae with a large blastopore (Fig. 1a). The embryos rotate inside their fertilization envelopes due to the action of the cilia that cover their surface. They are negatively buoyant, and after dissection from their envelopes, the embryos swim across the substratum or in the overlying water, propelled by their cilia. Subsequently the gastrulae elongate and a blunt projection forms at the anterior region, marking the formation of the preoral lobe and the future position of the central brachium (Figs. 1b, 2a). The blastopore narrows to a slit and closes about 2 days after fertilization. As the blastopore closes, two lateral folds appear in the preoral lobe 3 days after fertilization (Fig. 1c). These folds are pressed against the internal surface of the fertilization envelope and mark the formation of the two lateral brachia. The embryos are now early brachiolariae with three recognizable arms, or brachia (Figs. 1d, 2b). The central adhesive disk also begins to form (Fig. 2c). The larvae occupy all the space within the envelope and their cilia propel the surrounding fluid across their epithelium. Removal of 3-day-old larvae (\bar{X} = 500 μ m, length; SE = 4.9; n = 6) from the fertilization envelope reveals the presence of the large central brachium (\bar{X} = 174 μ m, length; SE = 7.1, n = 6) flanked by two small lateral brachia (\bar{X} = 124 μ m, length; SE = 4.6, n = 6). The body-to-arm-length ratio of the central arm is 2.9:1; that of the lateral arms is 4:1. The early larvae of *P. exigua* are similar to the late brachiolaria of *Patiriella* species that have planktonic development: all have one long central brachium and two short lateral brachia (Table II). The relative sizes of the brachia of unhatched *P. exigua* are particularly similar to the central and lateral arms of the lecithotrophic brachiolariae of *P. calcar* and *P. gunnii* (Table II).

The hatching process begins on the fourth day of development (Fig. 1e). This takes several days and involves softening of the fertilization envelope, which presumably results from hatching enzyme activity. During hatching the envelope is stretched and torn by movement of the

Table I

Chronology of development of Patiriella exigua at 19–21°C

Time	Stage
0	Fertilization
10 h	Blastula
15 h	Wrinkled blastula
24 h	Gastrula with large blastopore and spacious archenteron
2 days	Gastrula elongates along archenteric axis to form future preoral lobe; blastopore almost closed; anterior coelom present
3 days	Early brachiolaria, with 1 long central arm and 2 short lateral arms; each arm is occupied by a branch of the anterior coelom; adhesive disk forms; gut rudiment closes off from coelom; left and right posterior coeloms present
4 days	Hatching process begins with softening of fertilization envelope; brachiolaria have 3 arms equal in length; left and right posterior coeloms separate from anterior coelom and meet below gut; posterior coeloms are partially confluent; gut closed off; left and right hydrocoels present
5–6 days	Hatched brachiolaria: arms and adhesive disk well-developed; arms muscular and starting to become translucent; hydrocoel lobes form; hydropore opens; right hydrocoel pinches off and becomes filled with cells; ventral horn fuses with anterior coelom; skeletal spicules present; the larvae become pentamerous as adult arm rudiments become visible externally
6–7 days	Late brachiolaria: arms translucent; left posterior coelom spacious and right posterior coelom elongate; terminal and interradial ossicles present
8 days	Metamorphosis begins with flexure of the larval body and initiation of resorption of the larval arms and anterior coelom; hydrocoel lobes evident on oral surface
9–10 days	Larvae are stellate, first tube feet present; the anterior coelom closes; fused posterior coeloms on aboral side of gut; ventral horn on oral side of gut
11–12 days	Brachia reduced to stumps around adhesive disk; disk resorption initiated; two pairs of tube feet present in each radius; all primary skeletal plates present
13–14 days	Disk reduced to a plug in mouth region; eye spots present on terminal tube feet
15–18 days	Mouth opens

arms. Differential growth of the lateral brachia results in the formation of three arms equal in length. When removed from their fertilization envelope, the larvae adhere weakly to the substratum or swim away into the overlying water.

Hatching occurs 5–6 days after fertilization and, by this time, the brachia are noticeably muscular and extend through breaks in the envelope to attach to the substratum. Hatched brachiolariae (\bar{X} = 566 μ m, length; SE = 12.1; n = 9) have a well-developed brachiolar complex composed of three brachia equal in length (\bar{X} = 243 μ m; SE = 6.4; n = 9) and a large central adhesive disk (Figs. 1e; 2d). The body-to-arm-length ratio of these larvae is

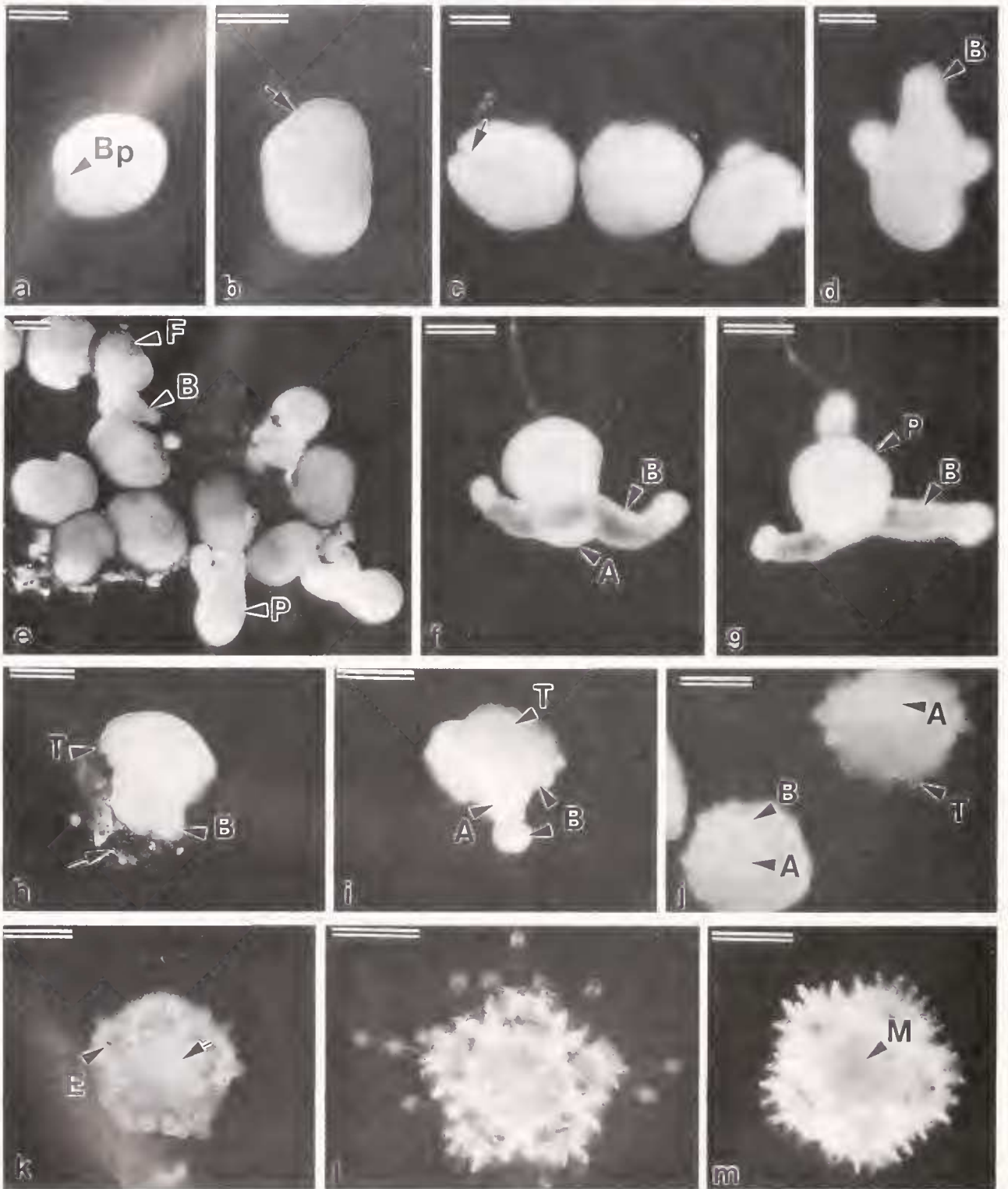


Figure 1. Light microscopy of development of live specimens. Scale bars = 200 μm . (a) Twenty-four hours: slightly elongate gastrula, dissected from the fertilization envelope, swimming in the culture dish. Bp, blastopore. (b) Thirty hours: left side of an elongate gastrula, dissected from the fertilization envelope, with a protrusion (arrow) at its anterior end marking formation of the preoral lobe. (c) Fifty-five hours: early

2.3:1. The brachiolariae resemble tripods, with the three muscular arms supporting the spherical posterior region. Growth of the brachia continues after hatching. They become more muscular and are translucent along their length, with bulbous tips (Figs. 1f, g; 2d, f). With these features, the larval arms bear a striking resemblance to adult tube feet. The adhesive disk is a large flat structure ($\bar{X} = 123 \mu\text{m}$; diameter, $\text{SE} = 3.1$, $n = 4$) occupying most of the width of the preoral lobe (Figs. 1f, g; 2f). The entire preoral portion ($243 \mu\text{m}$, length; $\text{SE} = 6.4$, $n = 9$) of the larvae consists of the hypertrophied brachiolar complex with which they adhere tenaciously to the substratum (Fig. 1g). Raised nodules of epithelial cells covering the tips of the arms and the disk may be composed of adhesive-secretory cells (Fig. 2e, f). Fragments of the fertilization envelope remain attached to the larvae for a variable amount of time after hatching (Fig. 1h). Before or during hatching, the hydropore opens as a shallow invagination on the left side of the plane of bilateral symmetry (Fig. 2d). The posterior region of the body starts to become pentamerous as the adult arm rudiments become visible externally (Fig. 1g).

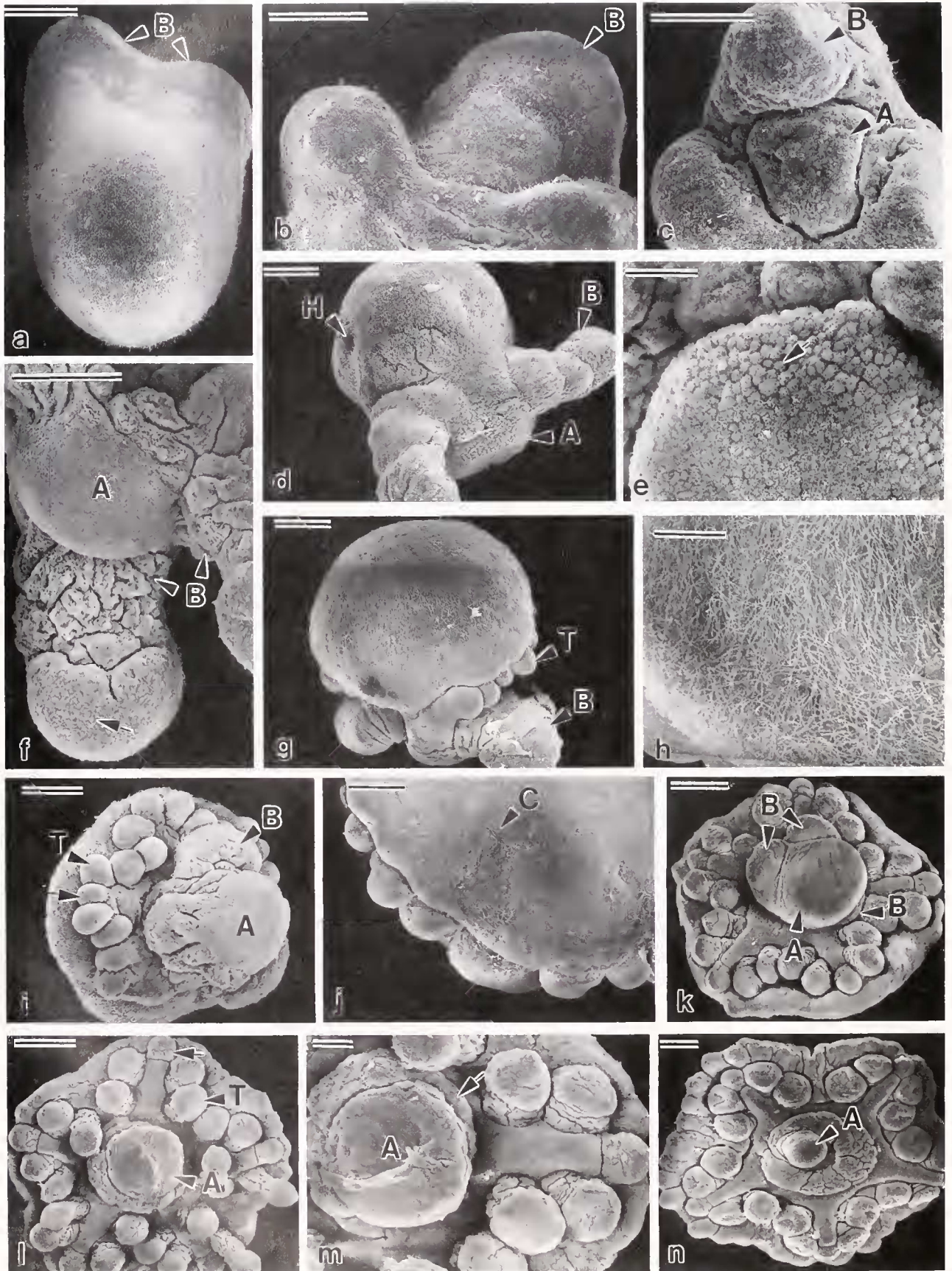
After hatching, the larvae usually remain in one place, but often rock back and forth on their arms. They occasionally move by attaching and detaching their arms. Occasionally, the larvae attach the adhesive disk to the substratum. In response to a strong current the brachia stretch in the direction of flow, and only concentrated flow from a pipette will dislodge the larvae. Late brachiolaria, 7 to 8 days old, have a mean length of $690 \mu\text{m}$ ($\text{SE} = 14.6$, $n = 7$) with three arms each $310 \mu\text{m}$ in length ($\text{SE} = 10.3$, $n = 7$). The ratio of body to brachium is 2.2:1, similar to that for the central brachia of *P. calcar* and *P. gunnii* (Table II). Compared with unhatched *P. exigua*, the lateral brachia of the late larvae have about doubled in length (Table II).

On about the 8th day of development, metamorphosis starts with flexure of the larval body to the left side and flattening of the body to mark the future oral and aboral surfaces (Figs. 1h, 2g). The larvae have a distinct angular, pentamerous shape. Four lobes of the hydrocoel appear

at the base of the preoral lobe on the future oral surface. A gap between lobes one and four is occupied by the preoral portion of the larva. If the larvae are placed on their aboral surface, they remain in this position and creep across the substratum, propelled by their cilia (Fig. 2h). Resorption of the brachiolar complex also starts on day 8 and is a gradual process that occurs concomitant with development of the tube feet (Figs. 1i–k; 2g, i). Nine-day-old metamorphosing larvae look like yolky stars and have the first pairs of tube feet present in four ambulacra (Figs. 1i, 2i). These tube feet move across the surface while the larvae remain attached to the substratum by the adhesive disk. By days 10 or 11, the brachia are reduced to small stumps around the base of the disk (Figs. 1j, 2k). The ciliary cover of the aboral surface is also reduced (*cf.* Fig. 2h, j). As the arms are resorbed, the adhesive disk plays a major role in attachment and the fifth hydrocoel lobe appears on the oral surface. When the tube feet associated with the fifth hydrocoel lobe develop, the positions of the five adult ambulacra are apparent (Figs. 1k; 2k, l). By day 12, resorption of the adhesive disk is under way as the tube feet take over the role of benthic attachment (Figs. 1k; 2l–n). In most larvae all traces of the larval arms are gone. By the time the disk is reduced to a plug in the mouth region of 13-day-old larvae, eye spots are present on the terminal tube feet (Figs. 1k; 2l–n). The tube feet now function in benthic attachment and, when placed on their aboral surface, the young stars are able to right themselves. The disk remains as a plug in the mouth region for several days until the mouth opens 15 to 18 days after fertilization (Fig. 1m). Metamorphosis is now complete and the juveniles extrude their stomach to feed. Yolk reserves present in the gut give the young stars a yellow color for several weeks. When these reserves are exhausted, the juveniles are virtually transparent and the gastric lobes and anal openings are evident.

Juvenile *P. exigua* were maintained in the laboratory for several months. They exhibited negative geotaxis, climbing the walls; upon reaching the air-water interface, they attached their tube feet to the surface of the water

Figure 1. (Continued) brachiolaria with folds at anterior end (arrow) that mark the formation of the larval arms. (d) Dorsal view of a swimming early brachiolaria dissected from envelope. B, brachium. (e) Five-day-old hatching brachiolariae. B, brachium; F, fertilization envelope; P, posterior region. (f) Six-day-old hatched brachiolaria. A, adhesive disk; B, brachium. (g) Seven-day-old late brachiolaria resemble tripods supported by tube-foot-like arms (B). The posterior region (P) is beginning to become pentamerous. (h) Side view of a 10-day-old metamorphosing larva. The brachia (B) are being resorbed and the larval body has flexed. The first tube feet (T) have formed. Arrow, remnant of fertilization envelope. (i) Ten-day-old metamorphosing larva viewed from the future oral surface of the juvenile. The adhesive disk (A) attaches the larva to the substratum as the tube feet (T) form. B, resorbing brachia. (j) Oral surface of two metamorphosing larvae—one (bottom) with resorbing brachia (B) and adhesive disk (A), and one (top) with the disk reduced to a plug in the mouth region. T, tube foot. (k) Fourteen days: the disk has been resorbed (arrow) and eye spots (E) are present on the terminal tube feet. (l) Seventeen days: aboral surface of juvenile. (m) Seventeen days: oral surface of juvenile with mouth (M) open.



and floated attached by surface tension for periods from hours to days.

Internal morphogenesis

The chronology of internal morphogenesis of *P. exigua* is outlined in Table 1. Gastrulae (24 h) have a spacious archenteron and the blastocoel is a narrow space in which mesenchyme cells are evident (Fig. 3a). A single coelom forms at the anterior end of the archenteron of 2-day-old elongate gastrulae and grows to occupy most of the anterior preoral region (Fig. 3b). This coelom subsequently forms the lumen of the central brachium. By the time the three brachial rudiments are evident externally in 3-day-old larvae, the anterior coelom has branched into the two lateral arms. The right and left posterior coeloms are also present in 3-day-old larvae as elongate extensions of the anterior coelom on either side of the gut (Fig. 3e). The gut wall has thickened and starts to close off from the coelom (Fig. 3c). During days 3 and 4, the right and left coeloms extend posteriorly and meet below the gut (Fig. 3c). These two coeloms become partially confluent and, depending on the plane of section, appear either as a single coelomic space or as two spaces separated by a mesentery. During the fourth day of development, the left and right posterior coeloms separate from the anterior coelom (Fig. 3d). On the left side the anterior coelom gives rise to the hydrocoel (Fig. 3d–f). The equivalent structure on the right side (Fig. 3d, e) corresponds to what MacBride (1896) termed the right hydrocoel in *Asterina gibbosa*. As in *A. gibbosa*, this structure subsequently buds off and the lumen becomes occluded by cells (Fig. 3f). Skeletal spicules appear as birefringent rods late on the fourth day or during the fifth day of development (Fig. 4a).

By the time the hatching process is near completion, on day 5, all five hydrocoel lobes have formed and the hydropore opens *via* the ciliated hydroporic canal (Fig.

3e). On days 5–6, the left posterior coelom gives rise to the ventral horn, which grows anteriorly to meet and fuse with the right anterior coelom (Fig. 3g, h). Prior to fusion, a mesentery separates the ventral horn and the anterior coelom (Fig. 3g). In 7-day-old larvae, the left posterior coelom is spacious and the right posterior coelom is a narrow coelomic space along the gut (Fig. 3i). A minute coelomic vessel appears between the left posterior coelom and the gut (Fig. 3i) and may represent the oral coelom described in *A. gibbosa* (MacBride, 1896), in which it forms the adult peripharyngeal coelom. The origin and fate of this structure in the ontogeny of *P. exigua* could not be determined. By the sixth day of development, terminal and interradianal ossicles can be identified. The terminals are small fenestrated plates positioned in the adult arm rudiments (Fig. 4b).

In 8-day-old metamorphosing larvae, the anterior coelom becomes closed off and is resorbed along with the brachiolar complex. Most of the primary skeletal elements viewed through the aboral surface have grown to form fenestrated plates, and additional spicules—including the terminal spines—have formed (Fig. 4c). The posterior region of 9-day-old larvae takes on the stellate appearance of the juvenile with formation of the tube feet and identifiable oral and aboral regions (Fig. 3k). The right posterior coelom lies along the aboral side of the gut, and the left posterior coelom/ventral horn lies along the oral side of the gut (Fig. 3j, k). On the aboral side of 11-day-old larvae, five terminal plates, five interradianal plates, and one central plate are identifiable. On the oral side, five pairs of elongate oral plates are present, one on each side of the radial canal, and five pairs of triradiate ambulacral spicules are present at the base of the tube feet. The marginal plates are also present. The next ossicles to appear on the aboral side, on about day 12 of development, are the radials (Fig. 4d).

Figure 2. Scanning electron microscopy of external development. Scale bars: Fig. 2a–d, f, g, i, k, l = 80 μ m; Fig. 2e, h = 25 μ m; Fig. 2j, l, m = 50 μ m. (a) Right side of an early brachiolaria showing development of the preoral lobe and arm rudiments (B). (b) Ventral view of the preoral region and arm rudiments (B) of an early brachiolaria. (c) Ventral view of the preoral region showing the developing adhesive disk (A) positioned between the three brachia (B). (d) Left side of a hatched brachiolaria with well-developed arms (B) and adhesive disk (A). H, hydropore. (e) Detail of adhesive disk showing the raised knobs of epithelial cells (arrow). (f) Detail of the brachiolar complex one day after hatching, showing the contracted tube-foot-like appearance of the brachia. The tips of the brachia (B) are covered by raised knobs of epithelial cells (arrow). A, adhesive disk. (g) Metamorphosing larva with body flexed. B, brachium; T, tube foot. (h) Ciliary cover of the future aboral surface of the juvenile. (i) Oral surface of a metamorphosing larva. tube feet (T) are present in four radii. The brachia are being resorbed while the disk (A) maintains benthic attachment. Arrow, terminal tube foot. (j) As metamorphosis continues, the ciliary (C) cover of the future aboral surface of the juvenile is reduced. (k) A metamorphosing larva viewed from the future oral surface. The larval arms (B) are reduced to small protrusions around the disk (A). Two pairs of tube feet are present in each radius. (l) Advanced metamorphosis: the disk (A) is reduced to a plug in the mouth region. T, tube foot; arrow, terminal tube foot. (m) Detail of resorbing disk (A) and one arm. The rim (arrow) surrounding the disk is the developing mouth. (n) Resorption of the disk (A) is nearly complete.

Table II

Body and arm dimensions of the brachiolaria larvae of *Patriella* species (SE, n)

Species	Larva	Mean length larval body (μm)	Length central brachium (μm)	Length lateral brachia (μm)	Ratio L:CB	Ratio L:LB
<i>Patriella regularis</i>	Planktotrophic brachiolaria	1204 (250, 7)	331 (61, 7)	100 (32, 7)	3.6:1	12:1
<i>Patriella calcar</i>	Planktonic lecithotrophic brachiolaria	590 (14.5, 8)	293 (18.7, 8)	141 (3.5, 8)	2.0:1	4.2:1
<i>Patriella gunnii</i>	Planktonic lecithotrophic brachiolaria	490 (23.4, 12)	236 (14.3, 12)	120 (4.9, 12)	2.1:1	4.1:1
<i>Patriella exigua</i> (Unhatched, 3 day)	Benthic lecithotrophic brachiolaria	500 (4.9, 6)	175 (7.2, 6)	124 (4.6, 6)	2.9:1	4.0:1
<i>Patriella exigua</i> (Hatched, 8 day)	Benthic lecithotrophic brachiolaria	690 (14.6, 7)	310 (10.3, 7)	310 (10.3, 7)	2.2:1	2.2:1

CB, central brachium; L, total larval length; LB, lateral brachium.

By the time the mouth opens (15–18 days), rudiments of the pyloric caeca are present. The timing of completion of the digestive tract with formation of the anal opening is variable and is delayed until most of the yolk reserves are exhausted. The oral plates form a frame around the mouth region, and the terminals are prominent rectangular plates over the terminal tube feet (Fig. 4e). The skeleton grows by adding branches that anastomose, with eventual intercalation of the plates (Fig. 4f).

Discussion

The ontogeny of *Patriella exigua* exhibits many modifications that appear to be associated with the evolution of benthic and lecithotrophic development. The major changes associated with the evolution of benthic development are (1) the adhesive properties of the jelly coat that serve to keep the embryos attached to the substratum; (2) the hardening of the fertilization envelope to form a tough protective case around the embryos; (3) the delay in hatching until the brachiolaria stage, at which time the larval arms are sufficiently developed to take over the role of attachment; and (4) the hypertrophied growth of the brachiolar complex, which serves as a tenacious attachment device for the larvae throughout their development. In contrast to *P. exigua*, *Patriella* species with planktonic development hatch as gastrulae (Byrne, 1991). As in other asteroids with lecithotrophic development (Chia, 1968; Strahmann, 1978, 1988; Chia and Walker, 1991), in *P. exigua* the acquisition of a large egg and the independence of an exogenous food source are considered to be derived features (Byrne, 1991). Compared with the planktotrophic development of *P. regularis* (Byrne and Barker, 1991), the major modifications associated with the shift to lecithotrophy by *P. exigua* are (1) the absence of the bipinnaria larva and ciliated bands; (2) the early closure of

the blastopore; and (3) the failure of the archenteron to connect with a stomodeum. The larval gut becomes a closed structure that serves as the rudiment for the adult gut. The transient appearance of a larval stomodeum and anus, described for *Asterina gibbosa* (MacBride, 1896; Marthy, 1980), was not observed in *P. exigua*.

Larvae similar to the brachiolaria of *P. exigua* are also characteristic of several other species in the family Asterinidae. These include species that attach their eggs to the substratum and species that brood their young externally (MacBride, 1896; Komatsu *et al.*, 1979; Marthy, 1980). All of these asterinids hatch as brachiolaria (MacBride, 1896; Komatsu *et al.*, 1979; Marthy, 1980). Like *P. exigua*, *Asterina minor* and *A. gibbosa* abandon their deposited eggs and develop through modified benthic brachiolaria (MacBride, 1896; Komatsu *et al.*, 1979; Marthy, 1980). The diminutive sea star *A. phylactica* also deposits its eggs and has a modified benthic brachiolaria, but in this species the adult remains with the developing progeny (Marthy, 1980; Crump and Emson, 1983). *A. minor* has a tripod larva virtually identical to that of *P. exigua*, whereas *A. gibbosa* and *A. phylactica* have brachiolariae with an asymmetrical attachment complex. The bilobed brachiolar complex of these species, with one lobe larger than the other, appears to be unique in the Asteroidea (MacBride, 1896; Marthy, 1980). Examination of the detailed work of MacBride (1896) suggests that the large lobe of the brachiolaria of *A. gibbosa* corresponds to the central brachium of the early brachiolaria of *P. exigua*, while the smaller lobe may have resulted from fusion of the two lateral arms. This speculation requires verification through detailed comparison of the development of the anterior coeloms of *A. gibbosa* and *P. exigua*.

Benthic development is also characteristic of the arctic genus *Leptasterias* of the order Forcipulatida. *Leptasterias* includes species that deposit their egg masses and species

that release their eggs into an external brood space under the arms or into an internal brood space in the gut (Kubo, 1951; Chia, 1968; Hendler and Franz, 1982; Himmelman *et al.*, 1982; Strathmann, 1987). Adults of *L. ochotensis similispinis* remain with the eggs, which are attached to the substratum, and the embryos develop through a tripod brachiolaria similar to the larva of *P. exigua* (Kubo, 1951). Other *Leptasterias* species that keep their young within a brood spaces also have tripod brachiolaria (Chia, 1968; Hendler and Franz, 1982; Himmelman *et al.*, 1982; Strathmann, 1987). Like *P. exigua*, these *Leptasterias* species produce young that hatch at the brachiolaria stage (Kubo, 1951; Chia, 1968).

Before they hatch, the larvae of *Patiriella exigua* are similar to the brachiolaria of *Patiriella* species with planktonic development because they have one long central brachium and two short lateral brachia (Table II). They are virtually identical to the early larvae of *P. calcar* and *P. gunnii* in the relative size of the brachia (Table II) and in their overall appearance and behavior. When dissected from their fertilization envelope, the larvae of *P. exigua* swim in the overlying water and, like the larvae of *P. calcar* and *P. gunnii*, have a uniform cover of cilia (Byrne, 1991; Byrne and Barker, 1991). Development of *P. exigua* through an early larval stage similar to the planktonic lecithotrophic brachiolariae of *P. calcar* and *P. gunnii* suggests that the tripod larval form results from differential growth of the lateral brachia. It appears that the evolution of benthic development by *P. exigua* involved modification of a lecithotrophic planktonic larva (Fig. 5). The delay in hatching from the early gastrula to the late brachiolaria stage would have been a parallel change for benthic life and presumably involves a delay in expression of the hatching enzyme. The robust brachia of *P. exigua* are more muscular than those of planktonic brachiolaria and are strikingly similar in structure and function to adult tube feet. The central adhesive disk also appears to be larger. This hypertrophic development of the brachiolar complex in *P. exigua* is undoubtedly an adaptation for permanent benthic attachment, as suggested for the benthic larvae of *Asterina minor* (Komatsu *et al.*, 1979).

Despite the taxonomic distance between *Leptasterias* and the Asterinidae, the similarity of their modified brachiolariae is striking (Chia, 1968; Komatsu *et al.*, 1979). In both *P. exigua* and *Leptasterias hexactis*, the early brachiolaria have one long and two short lateral brachia, while the late brachiolaria have arms that are equal in length (Chia, 1968). This suggests the possibility of convergence in the sequence of developmental changes associated with the evolution of benthic development in these two lineages. Considering that planktotrophic larvae are generally accepted as being ancestral in the Asterozoa (Strathmann,

1978), both groups probably had an ancestor with a planktonic lecithotrophic brachiolaria.

The possession of similar developmental patterns in asterozoans that abandon their deposited eggs and asterozoans that brood their young suggests that egg laying was an integral step in the sequence of reproductive and developmental changes that led to the evolution of brooding in the Asterozoa. Egg laying appears to be a preadaptive feature that made the shift to external brooding a relatively simple matter of the adult remaining with the egg mass, as seen in *Asterina phylactica* and several *Leptasterias* species (Chia, 1968; Hendler and Franz, 1982; Himmelman *et al.*, 1982; Crump and Emson, 1983; Strathmann, 1987). The converse could also occur, with loss of the brooding habit resulting in unattended egg masses.

In addition to the internal changes that result in the loss of larval feeding structures in *P. exigua*, there are also changes in coelomogenesis. The larvae of *P. exigua* form a single large anterior enterocoel instead of the paired anterior enterocoels and the single posterior enterocoel seen in *P. regularis* (Byrne and Barker, 1991). The changes in coelomogenesis are similar to those seen in other asterozoans with lecithotrophic brachiolaria (MacBride, 1896; Chia, 1968), including *P. calcar* and *P. gunnii* (Byrne, unpub). A small posterior enterocoel on the left side of the larval gut of *P. regularis* is a characteristic feature of the bipinnaria of asterinids, and recent work with *Asterina pectinifera* indicates that this enterocoel is the site of origin of the germ cells (Newman, 1925; Byrne and Barker, 1991; Inoue *et al.*, 1992). Although the presence of this posterior coelom in several planktotrophic asteroid larvae is suggested to result from heterotopy, similar to that described for the 'mesogen' of *Pteraster tesselatus* (Janies and McEdward, 1993), the original hypothesis—that it is a rudiment of a posterior coelom present in a common enteropneust-echinoderm ancestor—remains an alternative view (Gemmill, 1914; Byrne and Barker, 1991).

The timing of skeleton formation has also apparently been altered in the development of *P. exigua*: the first spicules appear early in the larvae, well before the start of metamorphosis. In contrast, the skeleton of *P. regularis* forms later in development, just before, and during, metamorphosis (Byrne and Barker, 1991).

Unlike the brachiolaria of *P. regularis*, in which the attachment complex takes up a relatively small portion of the preoral lobe (Byrne and Barker, 1991), the brachiolar complex of *P. exigua* is large and occupies most of the preoral portion of the larva. As a result, resorption of this substantial structure during metamorphosis is gradual, taking 6 to 7 days. The transformation from the larval to the juvenile form in *P. exigua* takes about half the duration of larval life. In contrast, *P. regularis* and the other species with planktonic development have a discrete settlement stage, and their metamorphic period oc-

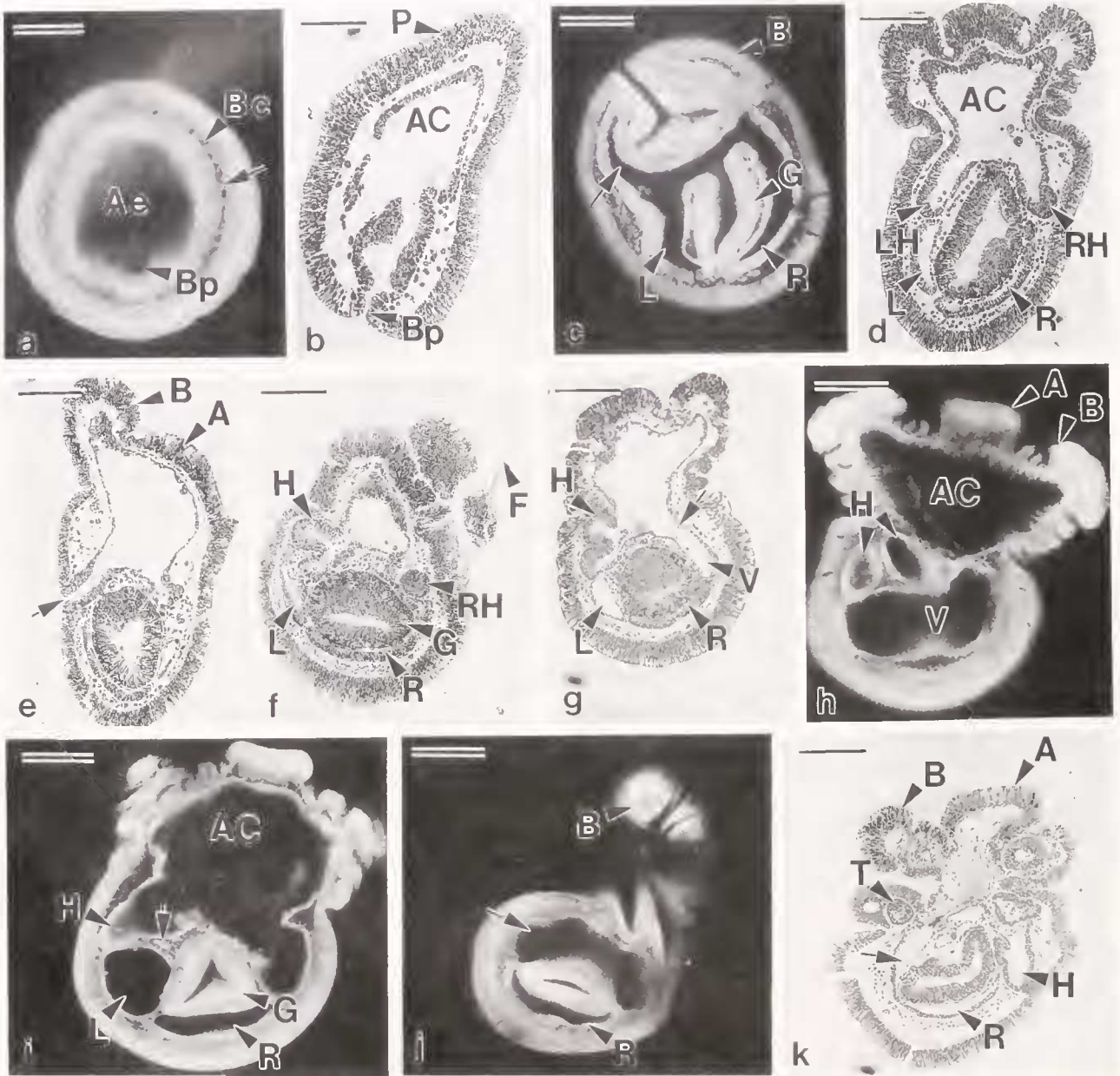


Figure 3. Confocal microscopy (Fig. 3a, c, h–j) and light microscopy (Fig. 3b, d–g, k) of internal development. Larvae are orientated to facilitate presentation of right and left structures. Scale bars = 100 μ m. (a) Thirty hours: early gastrula with large archenteron (Ae) and narrow blastocoel (Bc) containing mesenchyme cells (arrow). Bp, blastopore. (b) Fifty hours: sagittal section of an elongate gastrula/early brachiolaria dissected from its fertilization envelope, with the anterior coelom (AC) occupying the preoral lobe (P). Bp, blastopore. (c) Frontal section of a three-day-old early brachiolaria in fertilization envelope. The large ectodermal cleft at anterior end of larva arises as the brachia (B) are formed. Internally, the anterior coelom branches (arrow) into the three brachia. The left (L) and right (R) coeloms meet below the gut (G). Adjacent optical sections in the series show that the coelom is starting to separate from the gut. (d) Frontal section of a five-day-old brachiolaria. The left (L) and right (R) posterior coeloms have separated from the anterior coelom (AC). The blind extensions of the anterior coelom form the left (LH) and right (RH) hydrocoels. (e) Sagittal section of a five-day-old brachiolaria showing hydroporic canal opening (arrow) on dorsal surface. A, adhesive disk; B, brachium. (f) Frontal section of a hatching six-day-old brachiolaria with hydrocoel lobes (H), left (L) and right (R) posterior coeloms, and the detached right hydrocoel (RH) occluded by cells. F, fertilization envelope; G, gut. (g) Frontal section of a six-day-old brachiolaria showing ventral horn (V) fusing with anterior coelom. The two spaces are separated by a mesentery (arrow). H, hydrocoel lobe; L, left coelom; R, right coelom. (h) Frontal section of a seven-day-old brachiolaria showing the ventral horn (V) crossing the larva to connect with the anterior coelom (AC). Adjacent optical sections show that these two spaces have become confluent. A, adhesive disk; B, brachium; H, hydrocoel lobes. (i) Frontal section of a seven-day-old brachiolaria showing

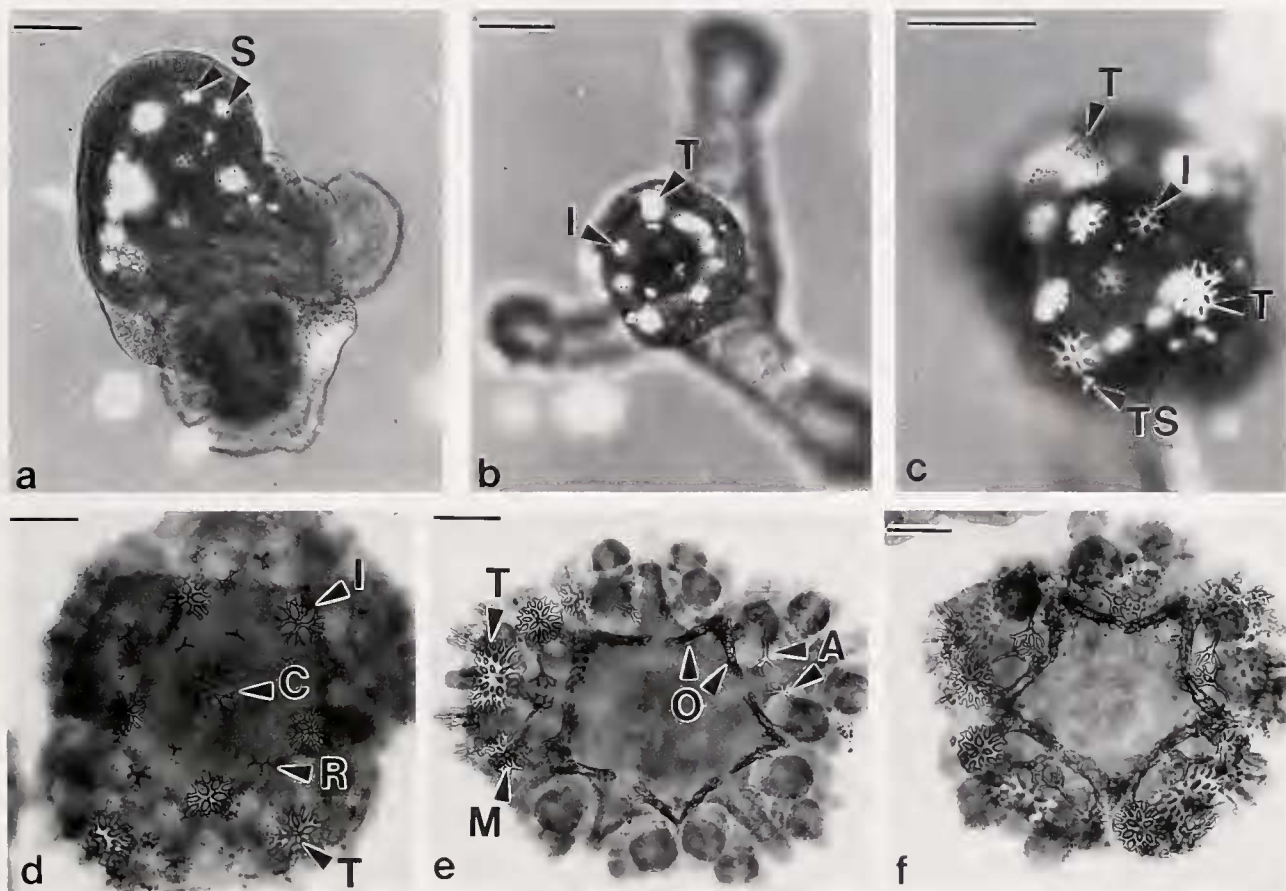


Figure 4. Development of the skeleton: a–c viewed with polarized light; d–f viewed with bright-field illumination. Scale bars = 100 μm . (a) The first spicules (S) appear in four-day-old brachiolaria. (b) Seven-day-old brachiolaria, the terminal (T) and interradial (I) ossicles are present. (c) Eight-day-old larva, the terminals (T) and interradials (I) are fenestrated plates. TS, terminal spine. (d) Ahoral surface of a 12-day-old metamorphosing larva showing the central (C), interradial (I) and terminal (T) plates and small radial (R) ossicles. (e) Oral surface of a 15-day-old metamorphosed larva. The orals (O) frame the mouth. A, ambulacral ossicle; M, marginal plate, T, terminal plate. (f) Intercalation of skeletal plates in juvenile.

cupies much less of their development time. Development of *P. regularis* takes 7–9 weeks, and the larval body is reduced to a wispy stalk within 2 days of the onset of metamorphosis (Byrne and Barker, 1991). Despite the difference in the size of the attachment complexes of *P. regularis* and *P. exigua*, the appearance of the adhesive surfaces is similar (Byrne and Barker, 1991). The adhesive disk and larval arms of *P. exigua* are covered by raised epithelial knobs which, in sectioned material, appear to be batteries of secretory cells, similar to those reported

for the brachiolaria of *Stichaster australis* and *Coscinasterias calamaria* (Barker, 1978; Byrne, pers. obs.). Resorption of the adhesive disk typically occurs after the brachia in asteroid metamorphosis, emphasizing the importance of disk adhesion prior to the formation of functional tube feet (Birkeland *et al.*, 1971; Barker, 1978).

The negative geotaxis and floating behavior of juvenile *P. exigua* are also reported for *P. pseudoexigua* and *Asterina minor* and are suggested to be a means of dispersal (Soliman and Nojima, 1984; Chen and Chen, 1992). In

Figure 3. (Continued) the spacious left coelom (L) and elongate right coelom (R). A small coelomic vesicle (arrow) is present between the gut (G) and anterior coelom (AC). (j) Radial section of a metamorphosing nine-day-old brachiolaria with flexed larval body. The right posterior coelom (R) lies on the aboral side of the gut and the left posterior coelom/ventral horn (arrow) lies on the oral side of the gut. (k) Radial section of a metamorphosing nine-day-old brachiolaria resorbing the brachia (B). A, adhesive disk; H, hydroporic canal; R, right posterior coelom; T, tube foot; arrow, left posterior coelom/ventral horn.

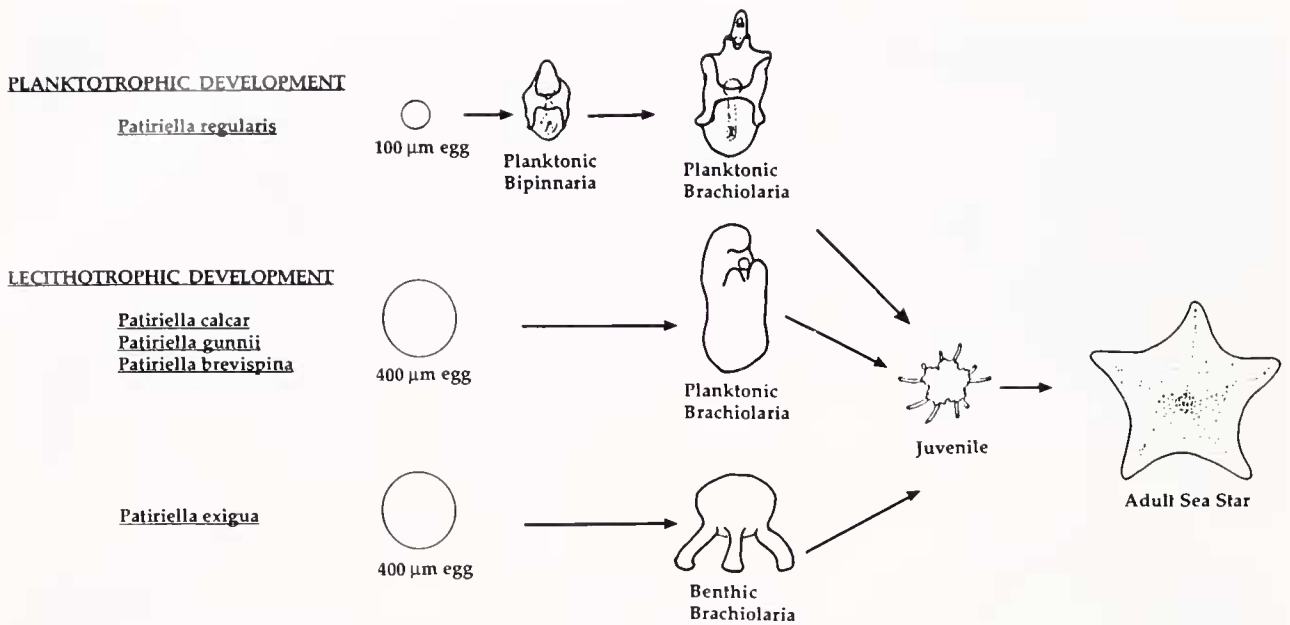


Figure 5. Diagram illustrating the developmental patterns of *Patiriella* species with the development of *P. regularis* (top) presumed to represent the ancestral state. The similarity of the early larva of *P. exigua* to the brachiolariae of *P. calcar* and *P. gunnii* (Table II) suggests that the tripod larval form and benthic development in *P. exigua* arose via an intermediate ancestor with pelagic lecithotrophy. The evolutionary changes in developmental pattern are suggested to be from planktotrophy to pelagic lecithotrophy to benthic lecithotrophy.

contrast, newly metamorphosed *P. calcar* and *P. gunnii* do not exhibit this floating behavior (Byrne, pers. obs.). *P. exigua* occupies shallow tide pools, and at low tide the adults are often covered by only a few millimeters of water with occasional emersion (Byrne, 1992). If juveniles in the field exhibit the same floating behavior observed in the laboratory, their attachment to the water surface during low tide may disperse them from the hatching area. *P. exigua* is widely distributed in southeastern Australia and is also found in South Africa, where its presence has been attributed to adult rafting and west-wind drift (Fell, 1962). The behavior of juvenile *P. exigua* indicates that transport of newly metamorphosed individuals may also be a means of dispersal. *P. exigua* lacks a planktonic larva, hence juvenile rafting may be an important mechanism for enhancing gene flow in this species. On the basis of field evidence, Highsmith (1985) suggested that juvenile rafting may account for the widespread distribution of species that lack a planktonic phase.

Comparison of the developmental chronologies of *P. exigua* and *P. regularis* reveals the embryological landmarks and structures that have been modified in the evolution of lecithotrophic development by *P. exigua*. Due to the presence of large yolk reserves, *P. exigua* has lost a feeding bipinnaria, a deletion that undoubtedly influences the duration of development in this species. In comparison with *P. regularis*, whose development is

dominated by the bipinnaria stage and takes 7–9 weeks, *P. exigua* has a dominant brachiolaria stage and development takes about 15 days. This difference is independent of temperature (Byrne and Barker, 1991). The similarity of the unhatched larvae of *P. exigua* to the brachiolariae of *P. calcar* and *P. gunnii* suggests that the shift to benthic development involved modification of a planktonic brachiolaria through the hypertrophy of the attachment structures. The evolution of benthic lecithotrophy by *P. exigua* also involved heterochronic changes such as the delay in hatching from the gastrula to the brachiolaria stage and the acceleration in the development of the juvenile form and the adult skeleton, the latter being similar to that described for echinoids with lecithotrophic development (Wray and Raff, 1990).

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